REMARKS

Claims 1, 2, 4-19 are pending in the present application.

Applicants wish to thank Examiner Kerr for the helpful and courteous discussion with their undersigned Representative on December 12, 2002, and for the suggestions to address the objections to the specification, the objections to the claims, and the rejections under 35 U.S.C. §112, second paragraph.

The rejection of Claims 1-2 and 6-8 under 35 U.S.C. §112, first paragraph, is obviated by amendment.

Claim 1 now recites "wherein the unmutated sequence of acetohydroxy acid synthase isozyme III is SEQ ID NO:2." Accordingly, this claim now provides clear structural limitations, as such these claims are adequately described in the present application. Applicants wish to acknowledge the Examiner for her indication, during the discussion with their undersigned Representative, that the amendments to Claim 1 "solve the issues concerning 112/first paragraph" (paper number 12).

Withdrawal of this ground of rejection is requested.

The rejection of Claims 3-8 under 35 U.S.C. §112, first paragraph, is obviated by amendment.

Applicants submit that with the present amendment, Claims 3 and 4 have been combined in such a way as to provide adequate written description for amended Claim 4. Applicants wish to acknowledge the Examiner for her indication, during the discussion with their undersigned Representative, that "Claims 3 and 4 should be combined to correct issues of 112/first paragraph" (paper number 12).

Withdrawal of this ground of rejection is requested.

The rejection of Claims 1 and 6 under 35 U.S.C. §102 over Smith et al is obviated by amendment.

Applicants submit that Smith et al fail to disclose or suggest presently amended Claim 1. In particular, Smith et al fail to disclose or suggest an isolated DNA molecule encoding a small subunit of acetohydroxy acid synthase isozyme III originating from *Escherichia coli* wherein the unmutated sequence of acetohydroxy acid synthase isozyme III is SEQ ID NO:2, much less any of the amendments thereto. The Examiner has recognized this deficiency in the disclosure of Smith et al by stating: "The art rejections will [sic] be obviated with the appropriate amendments as noted above" (paper number 12).

Applicants request withdrawal of this ground of rejection.

The rejection of Claims 3 and 6 under 35 U.S.C. §102 over Guardiola et al is obviated by amendment. Claim 3 has been canceled by the present amendment and request that this cancellation be entered without prejudice toward examination in an ensuing continuation application.

Applicants request withdrawal of this ground of rejection.

The rejection of Claims 1-2 and 4-8 under 35 U.S.C. §112, second paragraph, is obviated by amendment. Claims 1 and 4 have been amended in accordance with the Examiner's kind suggestion. As such, withdrawal of this ground of rejection is requested.

The rejection of Claims 4-5 under 35 U.S.C. §112, second paragraph, is obviated by amendment. Applicants have replaced the objected to phrase "delete a C-terminal region from the amino acid number 91 downwards" with "that replaces the glutamine residue at amino acid number 92 in SEQ ID NO: 2 with a stop codon" as supported by the specification at page 24, line 5. Withdrawal of this ground of rejection is requested.

The rejection of Claim 5 under 35 U.S.C. §112, second paragraph, is obviated by amendment. Applicants submit that the designation of amino acid 29 as an aspartic acid was inadvertent. Claim 5 and the specification have been amended to clarify this error. Support

for the present amended is offered by the remainder of the specification and SEQ ID NO:2 in the Sequence Listing. Withdrawal of this ground of rejection is requested.

The objection to the claims is obviated by amendment. Applicants have amended the claims as suggested by the Examiner. Withdrawal of this ground of objection is requested.

The objections to the specification based on the format and context of the Abstract, as well as for typographical errors therein, are obviated by amendment. Applicants have canceled the original Abstract appearing on page 37 and have replaced it with the attached substitute Abstract. Withdrawal of this ground of objection is requested.

The objection to the specification set forth in paper number 11, page 5, paragraph 10, is obviated by amendment. Applicants acknowledge the Examiner for identifying the inadvertent contradiction in nomenclature. As the Examiner has indicated, page 22 indicates the Asn29Tyr mutant is described as *ilvH3* and the Asn29Lys mutant is described as *ilvH4*. However, on page 24 the designation has been inadvertently reversed. In the present amendment, Applicants have corrected the text on page 24 to be consistent with that of page 22. In support of this amendment, Applicants submit herewith reference document 1, which is part of the experimental data of the present application. Accordingly, Applicants submit that the present amendment is proper. If the Examiner requires additional support, Applicants may provide reference document 1 and a further statement of its correlation to the present application in the form of a Declaration under 37 C.F.R. §1.132. Entry of the present amendment and withdrawal of this ground of objection is requested.

Applicants note that the Examiner has objected to the specification due to the confusing descriptions of the disclosed sequences and the drawings (paper number 11, page 4, paragraph 9). For the reasons set forth below, this objection in traversed in part.

Regarding Figure 1, pointing to page 21, the Examiner asserts that SEQ ID NOs: 5 and 6 are described in this figure. Applicants submit that the Examiner is mistaken with

respect to the description on page 21. For the Examiner's convenience, the relevant passage from page 21 is reproduced:

> "To obtain the mutant *ilvH* gene containing only one mutation: ^{14}Gly to Asp, the fact that this mutation creates a unique *MluI* site was utilized (Fig. 1). Thus, two primers having sequences depicted in SEQ ID NOs: 5 and 6 were synthesized."

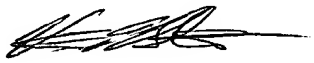
Evident from this passage, the sequence depicted in Figure 1 corresponds to the mutated site in the *ilvH* gene brought about due to the ^{14}Gly to Asp mutation. Accordingly, the figure does not show the primers utilized to effect this mutation. The primers are, in fact, what is shown in the Sequence Listing as SEQ ID NOs: 5 and 6. Therefore, Figure 1 and the description on page 21 are not at odds with one another, as such no amendment is believed to be necessary.

With respect to Figure 2, Applicants agree with the Examiner that the sequences disclosed in this figure have been mislabeled as SEQ ID NOs: 5 and 6. As the Examiner has properly pointed out, the sequence identifiers SEQ ID NOs: 7 and 8 refer to the sequences in Figure 2. Accordingly, Applicants submit herewith proposed changes to the drawings (Figure 2) to remedy this inadvertent error. If further correction and/or clarification is deemed necessary, Applicants kindly request that the Examiner contact their undersigned Representative to avoid unnecessary delays in the furtherance of examination of this application.

Applicants submit that the application is now in condition for allowance, and early notification of such action is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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IN THE SPECIFICATION

Please replace the paragraph beginning on page 24, line 3 with the following text:

--The mutation IlvH1 (^{17}Ser to Phe), *ilvH2* (^{14}Gly to Asp), *ilvH3* (^{29}Asn to [Lys] Tyr), *ilvH4* (^{29}Asn to [Tyr] Lys) and *ilvH612* (^{29}Asn to Lys and ^{92}Gln to a termination codon, TAG), conferred enzyme AHASIII resistance to L-valine inhibition as follows. That is, *E. coli* strain MI262 deficient of AHAS activity, after the introduction of the plasmids having various *ilvIH* genes showed the enzyme activity with different level of resistant to L-valine (Table 1). It can also be seen that AHAS from the strains containing pILVIH2 or pILVIH612 plasmids exhibits the highest level of resistance to L-valine.--

Please replace the paragraph beginning on page 5, line 21 with the following text:

--(5) The DNA of (4), wherein the mutation of the amino acid residue corresponding to serine residue at the amino acid number 17 is replacement of the serine residue with phenylalanine residue, the mutation of the amino acid residue corresponding to [aspartic acid] asparagine residue at the amino acid number 29 is replacement of the [aspartic acid] asparagine residue with lysine residue or tyrosine residue, and the mutation of the amino acid residue corresponding to glycine residue at the amino acid number 14 is replacement of the glycine residue with aspartic acid residue.--

Please replace the paragraph beginning on page 7, line 26 with the following text:

--The small subunit has a mutation to replace an amino acid residue corresponding to serine residue at the amino acid number 17 with another amino acid residue or a mutation to replace an amino acid residue corresponding to asparagine residue at the amino acid number

29 with another amino acid residue or a mutation to delete a C-terminal region from the amino acid number 91 downwards, in SEQ ID NO: 2, or a combination of two or more mutations selected from the group consisting of aforementioned mutations and a mutation to replace an amino acid residue corresponding to glycine residue at the amino acid number 14 with another amino acid residue in SEQ ID NO: 2. The small subunits of AHAS III which have these mutations also hereafter referred to as mutant small subunit of AHAS III. As the mutation, for the amino acid residue corresponding to serine residue at the amino acid number 17 is preferably exemplified by replacement of the serine residue with phenylalanine residue, and for the amino acid residue corresponding to [aspartic acid] asparagine residue at the amino acid number 29 it is exemplified by replacement of the [aspartic acid] asparagine residue with lysine or tyrosine residue, and for the amino acid residue corresponding to glycine residue at the amino acid number 14 it is preferably exemplified by replacement of the glycine residue with aspartic acid residue.--

IN THE CLAIMS

Please cancel Claim 3.

Please amend the claims as follows:

1. (Amended) [A] An isolated DNA [coding for] molecule encoding a small subunit of [actohydroxy] acetohydroxy acid synthase isozyme III originating from *Escherichia coli*, which [has a] mutation is selected from the group consisting of:

a) a mutation that replaces the serine [to replace an amino acid] residue [corresponding to serine residue] at [the] amino acid number 17 [with another amino acid residue] in SEQ ID NO: 2 with an amino acid other than serine and

b) a mutation that replaces [, or] both [of a mutation to replace an amino acid residue corresponding to] (i) the serine residue at [the] amino acid number 17 in SEQ ID NO: 2 with

an amino acid other than serine and [a mutation to replace an amino acid residue corresponding to] (ii) the glycine residue at the amino acid number 14 [with another amino acid residue] in SEQ ID NO: 2 with an amino acid other than glycine,

wherein the unmutated sequence of acetohydroxy acid synthase isozyme III is SEQ ID NO:2.

2. (Amended) The isolated DNA according to claim 1, wherein the mutation [of the amino acid residue corresponding to serine residue] at [the] amino acid number 17 [is replacement of the] replaces serine [residue] with a phenylalanine residue and the mutation [of the amino acid residue corresponding to glycine residue] at the amino acid number 14 [is replacement of the] replaces glycine [residue] with an aspartic acid residue.

4. (Amended) [The] An isolated DNA [according to claim 3, wherein the DNA codes for] encoding a large subunit and a small subunit of [actohydroxy] acetohydroxy acid synthase isozyme III originating from *Escherichia coli*,

wherein the small subunit [having a mutation] has a mutation that replaces the glycine residue at amino acid number 14 in SEQ ID NO: 2 with an amino acid other than glycine and has at least one mutation selected from the group consisting of:

a) a mutation that replaces the serine [to replace an amino acid] residue [corresponding to serine residue] at [the] amino acid number 17 [with another amino acid residue] in SEQ ID NO: 2 with an amino acid other than serine,

b) [or] a mutation [to replace] that replaces the asparagine [an amino acid] residue [corresponding to asparagine residue] at [the] amino acid number 29[with another amino acid residue] in SEQ ID NO: 2 with an amino acid other than asparagine, and

c) [or] a mutation [to delete a C-terminal region from the amino acid number 91 downwards,] that replaces the glutamine residue at amino acid number 92 in SEQ ID NO: 2 with a stop codon [, or a combination of two or more mutations selected from the group

consisting of aforementioned mutations and a mutation to replace an amino acid residue corresponding to glycine residue at the amino acid number 14 with another amino acid residue in SEQ ID NO: 2],

wherein the mutated acetohydroxy acid synthase isozyme III catalyzes the generation of (i) α-acetolactate from pyruvate and (ii) α-aceto-α-hydroxybutyrate from α-ketobutyrate and pyruvate; and is not inhibited by L-valine.

5. (Amended) The isolated DNA according to claim 4, wherein the mutation [of the amino acid residue corresponding to serine residue] at [the] amino acid number 17 [is replacement of the] replaces serine [residue] with a phenylalanine residue, the mutation [of the amino acid residue corresponding to aspartic acid residue] at [the] amino acid number 29 [is replacement of the aspartic acid residue] replaces asparagine with a lysine residue or a tyrosine residue, and the mutation [of the amino acid residue corresponding to glycine residue] at [the] amino acid number 14 [is replacement of the] replaces glycine [residue] with an aspartic acid residue.

6. (Amended) A bacterium which harbors the DNA according to [claims 1 or 3] claim 1 on chromosomal DNA or plasmid in said bacterium and has an ability to produce L-valine.

--10. – 19. (New)--

IN THE ABSTRACT OF THE DISCLOSURE

Please cancel the original Abstract appearing on page 37 and insert therefor the substitute Abstract submitted herewith as new page 37.

ABSTRACT

The present invention provides an isolated DNA molecule encoding a small subunit of acetohydroxy acid synthase isozyme III originating from *Escherichia coli* and mutants of *Escherichia coli* acetohydroxy acid synthase isozyme III, which are free from inhibition by L-valine an can catalyze the conversion of: (a) pyruvate to α-acetolactate and (b) α-ketobutyrate and pyruvate to α-aceto-α-hydroxybutyrate. The present invention also provides methods for producing L-valine by fermentation of a bacterium harboring the novel DNA molecule and/or expressing the mutant acetohydroxy acid synthase isozyme III.